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La Jolla, CA 92093

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14. ABSTRACT The rationale for using neurofeedback to affect changes in children on the autism spectrum is rooted in several assumptions. First, regions comprising the human mirror neuron system or MNS exhibit abnormal connections in ASD children. Second, the 8-13 Hz mu rhythm oscillations over sensorimotor cortex are functionally linked to the MNS network. Third, modifying these oscillation dynamics via neurofeedback training induces neural plasticity. Finally, normalization of abnormal connectivity is reflected in positive behavioral, cognitive, and electrophysiological changes. Our results show behavioral improvements following training in all the subcategories of the ATEC, a validated measure of the efficacy of an intervention. Similarly, we report improvement in the three subscales in adaptive measures, as measured by the Vineland Adaptive Behavioral Scale. Finally, social interactions during daily living were assessed with the Social Responsiveness Scale and improvements noted with training. BOLD activation results indicate that both TD and ASD groups have activity in IFG, IPL, and STS areas during imitation and in the observation of hand movement, but these activations are significantly greater for the TD group. Furthermore, our results are consistent with anatomical findings that show regions in the ASD brain exhibiting overconnectivity and underconnectivity compared to the TD brain. Finally, we show that NFT produces increases in MNS activity and reductions in abnormal functional connectivities between MNS areas compared to the TD brain.

15. SUBJECT TERMS
Quantitative electroencephalography (QEEG), neurofeedback, autism spectrum disorders

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ANNUAL TECHNICAL PROGRESS REPORT

INTRODUCTION:

This project is investigating the functional correlates of Autism Spectrum Disorder (ASD) with the goal of developing strategies to reduce cognitive, behavioral, and neurofunctional deficits. The primary goal is to test a model of the neural basis for changes in ASD induced by QEEG-guided plasticity-induced rehabilitation training. This intervention will help characterize the effects of altering cortical dynamics via operant conditioning of specific EEG frequencies on the amelioration of ASD symptoms and its impact on matched, typically developing children. It will help characterize the specific cognitive, behavioral, electrophysiological, and functional brain changes that occur with such training. The outcome will provide evidence for a link between "mirroring" activity in the human brain, EEG mu rhythms that reflect large-scale processing, and behaviors that comprise the core deficits in ASD. Furthermore, the project will help identify behavioral phenotypes that may contribute to diagnosis of the disorder and help predict successful treatment outcomes.

BODY:

1. IRB review of protocol changes: Prepare and submit changes to the currently approved protocol
Project received initial IRB approval on Aug 1, 2010. We have recently submitted the latest continuing review submission form on **04082012 .** No changes have occurred to the protocol.

2. Set up and test EEG protocols at study sites: Set up and test hardware and software for neurofeedback training at UCSD and protocols for neuroimaging at SDSU
EEG protocols, including hardware and software for neurofeedback training, were successfully installed and tested by the PI at the Cognitive Neuroscience Laboratory at UCSD. These continue to be active and problem-free. Likewise, approval of protocols for neuroimaging were obtained both at SDSU and UCSD and continue to be active and problem-free.

3. Training of research assistants: Begin training graduate student at UCSD and postdoc at SDSU
We have developed a rigorous program each academic quarter to train undergraduate students in the Cognitive Science program to serve as research assistants and neurofeedback trainers. It begins with a one-hour introduction to the program and the specifics of training. Students attend 3-5 training sessions in which they first observe and then practice the methodology on each other. This is followed by participation in actual training sessions with study participants. They are assigned to a senior trainer and over the course of 3-5 sessions learn to conduct the protocol on their own. This has worked quite well and has provided continuity in the training of participants. Currently there are 6-8 RAs who have received the training and are conducting actual neurofeedback sessions. Some were also trained to administer and score the behavioral, cognitive, and electrophysiological outcome measures in addition to administering neurofeedback training. Graduate student has been trained by the postdoc at SDSU on the administration and analysis of the neuroimaging protocols.

4. Training of students on PIRT: Graduate student will be responsible for training a set of undergraduate students who will help with the day-to-day training of participants
See #3 above

5. Advertise and recruit participants: Produce and distribute ads and flyers, as well as talk to various autism groups in San Diego

We have designed and utilize a number of flyers to advertise and recruit participants to the study. Most of our participants come from groups that we contact directly and provide information to. Additionally, we have subjects referred to us through the CARES of San Diego program and other groups associated with autism.

To date, we have recruited a total of 31 ASD and 15 TD participants to the study. Of that number a total of 29 participants (18 ASD and 14 TD) have been successfully scanned in the pre-training phase and 19 participants (11 ASD and 8 TD) have completed the post-training scans. Seven (7) ASD participants and 6 TDs dropped out without completing training. To improve patient accrual, we formed a partnership with the Speech and Language Development Center (SLDC) located in Buena Park, California to essentially duplicate our efforts there and increase patient accrual but progress has been slow. To date, we have trained their staff to carry out the assessments and outcome measures (both paper and pencil and electrophysiological tasks) as well as training for the actual neurofeedback procedure. Although SLDC has a readily available pool of children on the spectrum, who attend their school, as well as TD children the difficulty has been pre-screening them to include in the study.

6. Selection of participants (Clinical assessments: ADOS, ADI, WASI): Potential participants are evaluated by consultant to determine they meet inclusion criteria.
Our consultant, Dr. Alan Lincoln, Director of CARES in San Diego, and his staff are directly responsible for the clinical assessments, the scoring and interpretation of results. We have set up an efficient system to schedule participants in a timely manner.

7. Pre-training assessment: Schedule and administer cognitive, behavioral and electrophysiological assessments. These include: MSI, QEEG, TOVA, imitation, social-perceptual, social-cognitive, ATEC, neuroimaging
Participants visit the CARES center in San Diego for the initial clinical evaluation. Following that they visit the UCSD campus for the initial electrophysiological assessment (QEEG, MSI, TOVA, and paper and pencil questionnaires). Once those are completed they are scheduled for a neuroimaging scan at the Center for Functional MRI, W.M. Keck Building at UCSD.

We continue to gather electrophysiological outcome measures, including a *Quantitative EEG*, the *Mu Suppression Index (MSI)***,** and the *Emotion Discrimination Task (EDT).* We also continue to administer a set of pre- and post-training paper and pencil assessments, including the *Autism Treatment Evaluation Checklist (ATEC),* the *Vineland Adaptive Behavior Scales (VABS), and* the *Social Responsiveness Scale* (SRS)

We have made good progress in terms of gathering the neuroimaging data before and after training and beginning the analyses to determine whether NFT induces neural plastic changes and affects the brain's functional neuroanatomy. Some participants have required training with a mock scanner to desensitize them to the scanning equipment and some participants exhibit significant movement artifact as to necessitate excluding them from the analysis. The entire imaging session lasts approximately 1.5-hrs per participant and involves the following:

- 6-min echo planar imaging (EPI) resting state scan
- Three 5-min EPI task runs (the task is an imitation task)
- A spoiled gradient recalled (SPGR) high-resolution anatomical scan
- 8-min diffusion tensor imaging scan
- 3-slice magnetic resonance spectroscopy (MRS) scan

8. QEEG-guided PIRT (neurofeedback) training: Schedule and begin 30 hrs of training. This will require setting up parking areas for participants, arranging for a student to act as scheduler and point of contact with parents.
If participants meet the clinical criteria and complete the EEG and neuroimaging components then they are ready to begin the neurofeedback training. This requires a great deal of flexibility on our part to be able to accommodate children who are very busy during the school year and summer. We have found that an important component of this is to have one person entirely responsible for scheduling, as there is a significant amount of changes during the first few weeks. Once a schedule is found that works for parents and children it is usually kept throughout the rest of the training.

9. Post-training assessment: Following completion of 30 hrs of training, begin scheduling and administration of cognitive, behavioral, and electrophysiological assessments. These are the same tools used in the pre-training assessment (MSI, QEEG, TOVA, imitation, social-perceptual, social-cognitive, ATEC, neuroimaging)

The post-training assessments also require a great deal of scheduling and flexibility to make sure they are completed within a reasonable amount of time. We have worked out a system for scheduling all the assessments such that it only requires two additional visits.

10. Data processing and analysis

A significant amount of progress has been made in processing and analyzing data collected thus far, both in terms of behavioral, electrophysiological, and functional neuroanatomical results.

11. Preparation and submission of journal article(s)

We are currently preparing our first full-length journal submission (see Appendix).

12. Integration of project findings & preparation of R01 application

This is on our minds but remains for sometime in the near future.

KEY RESEARCH ACCOMPLISHMENTS:

- Participant recruitment to date (30 ASD and 14 TD) is on target
- Participants who have completed training (10 ASD and 4 TD)
- Participants currently undergoing neurofeedback training (2 ASD and 1 TD)
- Participants who were initially recruited but chose to drop out or were excluded due to problems (12 ASD and 5 TD)
- Neuroimaging scans have been completed for all pre-training participants (29)
- Neuroimaging scans completed for all post-training participants (10, but one is unusable)
- ***Preliminary analysis of functional neuroanatomy are consistent with predictions***
- We have established a partnership with the Speech and Language Development Center (SLDC) located in Buena Park, California (north of the UCSD campus) to essentially duplicate our efforts there to increase patient accrual.
- Personnel at SLDC who will administer clinical assessments have been identified, trained and certified.
- SLDC Research assistants who will administer and score outcome measures, electrophysiological tests and neurofeedback training have been trained.
- Paper and pencil assessments, software tools, and protocol design have been set up at SLDC
- Neurofeedback hardware and software implementation, protocols, testing of procedures, and training of personnel are completed and we have begun testing participants.

REPORTABLE OUTCOMES:

1. Datko, M., Müller, R-A., Pineda, J.A. Functional neuroanatomical changes produced by mu-based neurofeedback training in children on the autism spectrum, full-length manuscript, in preparation.
2. Datko, M. C., Pineda, J. A., Müller, R. A. Functional Neuroanatomical Changes Induced by Mu-based Neurofeedback Training in Children on the Autism Spectrum, IMFAR, Montreal, Canada, 2011.
3. Pineda, J.A., Datko, M. & Axel-Muller, R. Functional Neuroanatomical Changes Induced by Mu-based Neurofeedback Training in Children on the Autism Spectrum. CNS Conference, Chicago, 2012.
4. Datko, M., Carrasco, K., Müller, R-A. , Pineda, J.A. Changing the Dynamics of the Mirror Neuron System through Neurofeedback: Effects on ASD Behavior, Electrophysiology, and Functional Neuroanatomy, SABA presentation, 2012.

5. Pineda, J.A. Mirror Neurons, Mu Rhythms and Autism Spectrum Disorders. Developmental Psychobiology Conference, Hawaii, 2012.
6. Pineda, J.A. Functional Neuroanatomical Changes in the Mirror Neuron System Produced by Neurofeedback Training of Children on the Autism Spectrum. Mirror Neuron Conference, Erice, Italy, 2012.

CONCLUSION:

It is widely agreed that the autistic brain is characterized by widespread aberrant connectivity that could underlie abnormal social behaviors. Nonetheless, the nature of the brain's experience-dependent plasticity suggests that these abnormal connection patterns may be reversed with the proper type of treatment. Indeed, neurofeedback training (NFT), an intervention based on operant conditioning resulting in self-regulation of brain electrical oscillations, has shown promise in addressing marked abnormalities in functional and structural connectivity. The effects of 30 hours of mu-rhythm based NFT on children on the autism spectrum (ASD), as well as typically developing (TD) children, were assessed behaviorally, electrophysiologically, and with fMRI. Prior to training, ASD participants showed significantly less activation compared to TD controls in an object-directed imitation task in areas associated with the human mirror neuron system (hMNS). Following training, ASD participants showed significantly greater activation in this task while TD participants did not. Prior to training, ASD participants also showed both over- and underconnectivity in resting state functional connectivity between areas of the hMNS compared to TD participants. These differences were significantly reduced following NFT. The fMRI changes accompanied improvements in behavioral and electrophysiological assessments in the ASD but not TD participants, indicating that the positive benefits shown to result from NFT are accompanied by modifications in functional neuroanatomy.

An obvious limitation to these data is the small sample size. However, the results are surprisingly strong and clear in light of this caveat. Furthermore, it provides a very good preliminary set of results to leverage for an R01 grant. We anticipate a number of future analyses to be performed on the data obtained from this study. One important step will be to assess the degree of correlation between symptom severity and the magnitude of behavioral/neural response to NFT. This type of analysis will address the issue of whether mu neurofeedback is more beneficial for individuals with more severe symptoms of ASD. Another important question is whether those individuals with the most dramatic behavioral and parental assessment improvements also show the greatest changes in functional neurophysiological activation during the imitation task.

Our findings have implications for operationalizing the benefits of NFT towards practical solutions to the early diagnosis and possible repair of MNS deficits in autism. It is generally accepted that autism research should be translational. The increasing number of families affected by it represent a growing concern for society, with therapeutic approaches limited in efficacy but costly in time and money. Despite solid evidence for the importance of genetic factors in autism, numerous large-scale studies have failed to translate this into effective therapies. The reasons are many and imply that a comprehensive model of neurodevelopmental disturbances in autism may not become available for decades. However, children with ASD and their parents cannot wait, but rely on the immediate pursuit of treatment options that are promising. Techniques such as EEG have been used extensively to characterize functional brain abnormalities associated with ASD. While the current project will significantly contribute to this endeavor, its primary impact and importance derives from the application of these techniques to prediction and intervention. Cognitive, behavioral, and electrophysiological characterization before and after neurofeedback training allow us to develop phenotypic responses and therefore predictive measures to assess the success or failure of such interventions. NFT approaches appear to hold great promise for a large portion of children with ASD and this approach is particularly promising because it could combine QEEG analyses with an easy to use, ASD targeted in-home neurofeedback system. We believe that with such an approach many of the barriers to successful therapeutic intervention can be overcome. QEEG can be used to both identify ASD children likely to benefit from training, and profile the training protocol most likely to benefit an individual child. The in-home system provides an autism-specific, easy to use, comfortable therapeutic option that has the potential to improve compliance and the quality of data used for the feedback, increasing overall efficacy. Providing a personalized approach to this

type of intervention, which can be conducted primarily in the home, will substantially reduce costs in time and money for ASD families, while improving the potential for positive behavioral outcomes seen previously in laboratory studies.

REFERENCES:

1. R. A. Muller, *Ment. Retard. Dev. Disabil. Res. Rev.* **13**, 85 (2007).
2. M. A. Just, S. Varma, *Cogn Affect. Behav. Neurosci.* **7**, 153 (2007).
3. M. E. Villalobos, A. Mizuno, B. C. Dahl, N. Kemmotsu, R. A. Muller, *Neuroimage* **25**, 916 (2005).
4. D. E. Welchew *et al.*, *Biol. Psychiatry* **57**, 991 (2005).
5. V. L. Cherkassky, R. K. Kana, T. A. Keller, M. A. Just, *Neuroreport* **17**, 1687 (2006).
6. D. P. Kennedy, E. Courchesne, *Soc. Cogn Affect. Neurosci.* **3**, 177 (2008).
7. M. A. Just, V. L. Cherkassky, T. A. Keller, N. J. Minshew, *Brain* **127**, 1811 (2004).
8. E. Courchesne, K. Pierce, *Curr. Opin. Neurobiol.* **15**, 225 (2005).
9. D. P. Kennedy, E. Redcay, E. Courchesne, *Proc. Natl. Acad. Sci. U. S. A* **103**, 8275 (2006).
10. J. H. Williams, A. Whiten, T. Suddendorf, D. I. Perrett, *Neurosci. Biobehav. Rev.* **25**, 287 (2001).
11. G. di Pellegrino, L. Fadiga, L. Fogassi, V. Gallese, G. Rizzolatti, *Exp. Brain Res.* **91**, 176 (1992).
12. G. Rizzolatti, L. Craighero, *Annu. Rev. Neurosci.* **27**, 169 (2004).
13. J. A. Pineda, *Brain Res. Brain Res. Rev.* **50**, 57 (2005).
14. R. Mukamel, A. D. Ekstrom, J. Kaplan, M. Iacoboni, I. Fried, *Curr. Biol.* (2010).
15. M. Iacoboni *et al.*, *Science* **286**, 2526 (1999).
16. G. Hickok, *J. Cogn Neurosci.* **21**, 1229 (2009).
17. L. Turella, A. C. Pierno, F. Tubaldi, U. Castiello, *Brain Lang* **108**, 10 (2009).
18. N. Nishitani, S. Avikainen, R. Hari, *Ann. Neurol.* **55**, 558 (2004).
19. N. Hadjikhani, R. M. Joseph, J. Snyder, H. Tager-Flusberg, *Cereb. Cortex* **16**, 1276 (2006).
20. R. Bernier, G. Dawson, S. Webb, M. Murias, *Brain Cogn* **64**, 228 (2007).
21. M. Dapretto *et al.*, *Nat. Neurosci.* **9**, 28 (2006).
22. H. Theoret *et al.*, *Curr. Biol.* **15**, R84 (2005).
23. L. M. Oberman *et al.*, *Cognitive Brain Research* (2005).
24. M. Carpenter, K. Nagell, M. Tomasello, *Monogr Soc. Res. Child Dev.* **63**, i (1998).
25. S. Baron-Cohen, *Ann. N. Y. Acad. Sci.* **1156**, 68 (2009).
26. M. Dapretto *et al.*, *Nat. Neurosci.* **9**, 28 (2006).
27. L. M. Oberman, V. S. Ramachandran, J. A. Pineda, *Neuropsychologia* (2008).
28. R. Raymaekers, J. R. Wiersema, H. Roeyers, *Brain Res.* **1304**, 113 (2009).
29. E. L. Altschuler, A. Vankov, V. Wang, V. S. Ramachandran, J. A. Pineda, *J. Cogn Neurosci.* (1998).
30. G. Pfurtscheller, C. Brunner, A. Schlogl, F. H. Lopes da Silva, *Neuroimage.* **31**, 153 (2006).
31. S. D. Muthukumaraswamy, B. W. Johnson, N. A. McNair, *Brain Res. Cogn Brain Res.* **19**, 195 (2004).
32. D. J. McFarland, L. A. Miner, T. M. Vaughan, J. R. Wolpaw, *Brain Topogr.* **12**, 177 (2000).
33. D. Arnstein, F. Cui, C. Keysers, N. M. Maurits, V. Gazzola, *J. Neurosci.* **31**, 14243 (2011).
34. L. M. Oberman, V. S. Ramachandran, *Psychol. Bull.* **133**, 310 (2007).
35. T. Fuchs, N. Birbaumer, W. Lutzenberger, J. H. Gruzelier, J. Kaiser, *Appl. Psychophysiol. Biofeedback* **28**, 1 (2003).
36. D. J. Fox, D. F. Tharp, L. C. Fox, *Appl. Psychophysiol. Biofeedback* **30**, 365 (2005).
37. R. Coben, S. Sherlin, W. J. Hudspeth, K. McKeon, *Journal of Autism and Developmental Disorders* (2009).
38. J. A. Pineda *et al.*, *Research in Austism Spectrum Disorders* **2**, 557-581 (2008).
39. M. B. Sterman, *Biofeedback Self Regul.* **21**, 3 (1996).
40. S. R. Roth, M. B. Sterman, C. D. Clemente, *Electroencephalogr. Clin. Neurophysiol.* **23**, 509 (1967).
41. J. E. Walker, *Clin. EEG. Neurosci.* **39**, 203 (2008).
42. T. Ros, M. A. Munneke, D. Ruge, J. H. Gruzelier, J. C. Rothwell, *Eur. J. Neurosci.* **31**, 770 (2010).

43. M. Beauregard, J. Levesque, *Appl. Psychophysiol. Biofeedback* **31**, 3 (2006).
44. J. A. Pineda, *Behav. Brain Funct.* **4**, 47 (2008).
45. L. Q. Uddin, V. Menon, *Neurosci. Biobehav. Rev.* **33**, 1198 (2009).
46. S. J. Ebisch *et al.*, *Hum. Brain Mapp.* **32**, 1013 (2011).
47. M. Assaf *et al.*, *Neuroimage.* **53**, 247 (2010).
48. J. H. Williams *et al.*, *Neuropsychologia* **44**, 610 (2006).
49. S. H. Johnson-Frey *et al.*, *Neuron* **39**, 1053 (2003).
50. S. Avikainen, A. Wohlschlager, S. Liuhanen, R. Hanninen, R. Hari, *Curr. Biol.* **13**, 339 (2003).

APPENDICES:

1. Datko, M., Müller, R-A., Pineda, J.A. Functional neuroanatomical changes produced by mu-based neurofeedback training in children on the autism spectrum, full-length manuscript, in preparation. (see attachment of early draft)
2. IMFAR, Montreal, Canada, 2011.

Functional Neuroanatomical Changes Induced by Mu-based Neurofeedback Training in Children on the Autism Spectrum.

Datko, M. C.[1,2], Pineda, J. A.[1], Müller, R. A.[2]
1. Cognitive Neuroscience Laboratory, Cognitive Science Department, UCSD, La Jolla, CA
2. Brain Development Imaging Laboratory, Psychology Department, SDSU, San Diego, CA

Background: Autism Spectrum Disorders (ASD) may arise from atypical anatomical and functional connections and therefore have been characterized as a 'disconnection syndrome'. Impaired connectivity may lead to desynchronization and ineffective intra- and interhemispheric communication in neural circuits affecting higher order cognitive processes. While no single explanation can account for the ASD profile, converging evidence implicates the human mirror neuron system (MNS). Studies from our laboratory have shown that ASD individuals exhibit normal EEG mu rhythm suppression for self-generated movement but fail to suppress during observation of movement compared to typically developing (TD) controls. On the other hand, suppression is normal if the actors being observed are familiar, suggesting that the MNS is not entirely broken. We have shown that significant improvement occurs in social engagement and related behaviors, as well as in the electrophysiology of ASD children following neurofeedback training focused on the mu-rhythm.
Objective: The present study tested whether functional and structural neuroanatomical changes occur after 20 weeks of mu-based neurofeedback training.
Methods: Neurofeedback training is an operant conditioning task in which trainees learn to control mu rhythm (8-13 Hz) power at electrode site C4, over the sensorimotor cortex in the right hemisphere. Games and movies on a computer reward increased mu-power and decreased muscle activity. All participants complete 30 hours of this training (45 min/session x 2 sessions/week x 20 weeks). Prior to and again immediately following training, participants underwent fMRI scans that included the following protocols: resting state fMRI (6 min), 3 fMRI runs of a task that involved imitation and observation of object-oriented finger movements (total of 15 min), anatomical (5 min), and diffusion tensor imaging (10 min). Contrary to imitation tasks previously used by Iacoboni (1999) and Williams (2006), the imitation task in our study was object-oriented (pressing buttons on a button-box). Diffusion tensor imaging data were collected to assess white matter changes associated with neurofeedback in pathways connecting areas of the MNS.
Results: Before training, greater activation occurred in regions of interest related to MNS in TD compared to ASD during object-oriented imitation and observation. These areas of differential activation included left inferior frontal gyrus (IFG) and bilateral inferior parietal lobules. Abnormal resting state functional connectivity (both under- and over-connectivity) between MNS regions of interest was also seen in ASD compared to TD groups. Altering mu rhythm dynamics with training was found to result in increased activity in the IFG and other relevant MNS areas, as well as normalization of functional connectivity in the MNS circuits in ASD children.
Conclusions: These preliminary data indicate plasticity within the mirror neuron system occurs in response to mu-based neurofeedback training in ASD. Both activation and connectivity measures were found to normalize with training.
Funding: Department of Defense (DOD) Autism Research Program of the Office of the Congressionally Directed Medical Research Programs (CDMRP) to J.A.P.

3. CNS Conference, Chicago, 2012.

Functional Neuroanatomical Changes Induced by Mu-based Neurofeedback Training in Children on the Autism Spectrum. Pineda, J.A.[1,2], Datko, M.[1] & Axel-Mueller, R.[3] Cognitive Science Department[1] and Group in Neurosciences[2], UCSD, Psychology Department[3], San Diego State University, San Diego, CA. Autism Spectrum Disorders (ASD) may arise from atypical anatomical and functional connections and therefore produce abnormal activity among different regions of the brain. This type of 'disconnection syndrome' could lead to desynchronization and ineffective intra- and interhemispheric communication in neural circuits affecting higher order cognitive processes. While no single explanation can account for the ASD profile, converging evidence implicates the human mirror neuron system (MNS). Studies from our laboratory have shown that ASD individuals exhibit normal EEG mu rhythm suppression for self-generated movement but fail to suppress during observation of movement compared to typically developing (TD) controls. On the other hand, suppression is normal if the actors being observed are familiar, suggesting that the MNS is not entirely broken. We have shown that significant improvement occurs in social engagement and related behaviors, as well as in the electrophysiology of ASD children following neurofeedback training focused on the mu-rhythm. The present study tested whether functional neuroanatomical changes occur after mu-based neurofeedback training (45 min x 2 week x 20 weeks). Before training, greater activation occurred in regions of interest related to MNS in TD compared to ASD during object-oriented imitation and observation. Abnormal functional connectivity, under- and over-connectivity, were also present in ASD compared to TD groups among MNS areas. Altering mu rhythm dynamics with training was found to result in increased activity in the inferior frontal gyrus (IFG) and other relevant MNS areas, as well as normalization of functional connectivity in the MNS circuits in ASD children.

SUPPORTING DATA:

Fig. 1. Object-oriented Imitation task used during the neuroimaging part. Task adapted from Iacoboni (1999).

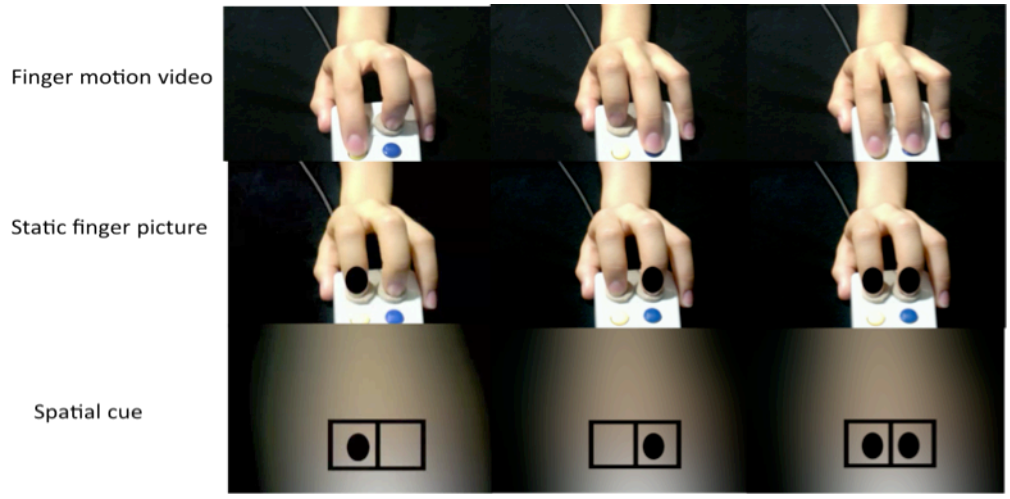


Fig. 2. Behavioral performance of ASD and TD groups in the object-oriented imitation task. Note there is little difference between groups.

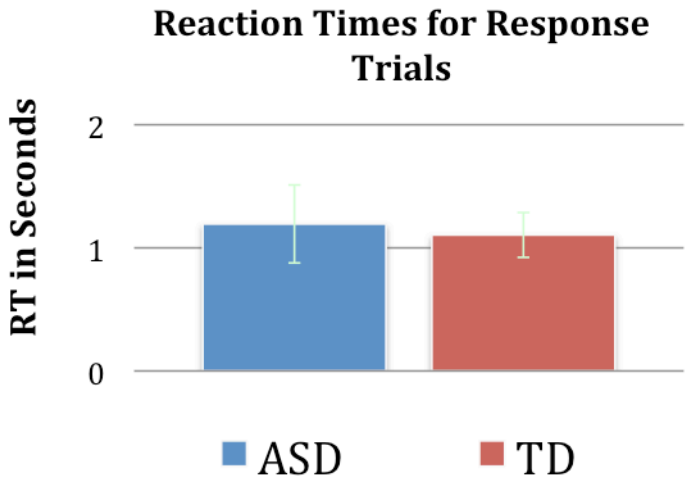


Fig. 3. Areas in the right inferior frontal gyrus and bilateral inferior parietal lobule showing greater activity in TD than ASD in the imitation task but not to the static hand or spatial cue pictures.

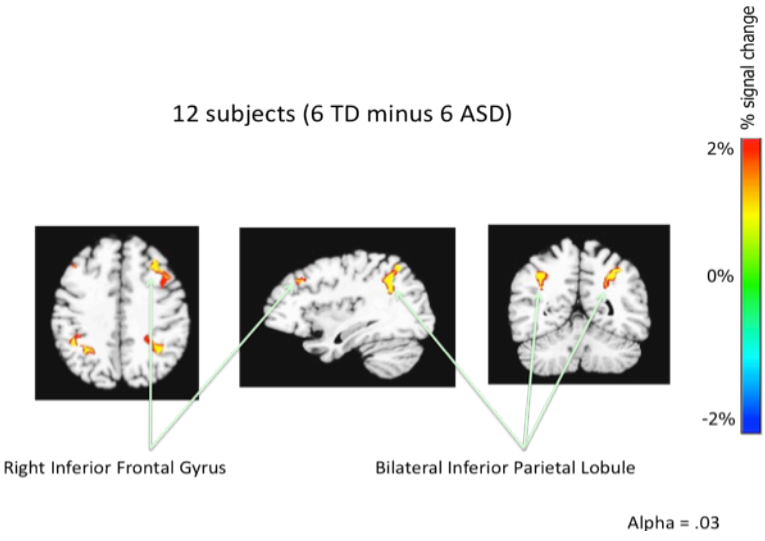


Fig. 4. Group correlation differences showing underconnectivity and overconnectivity in the ASD brain

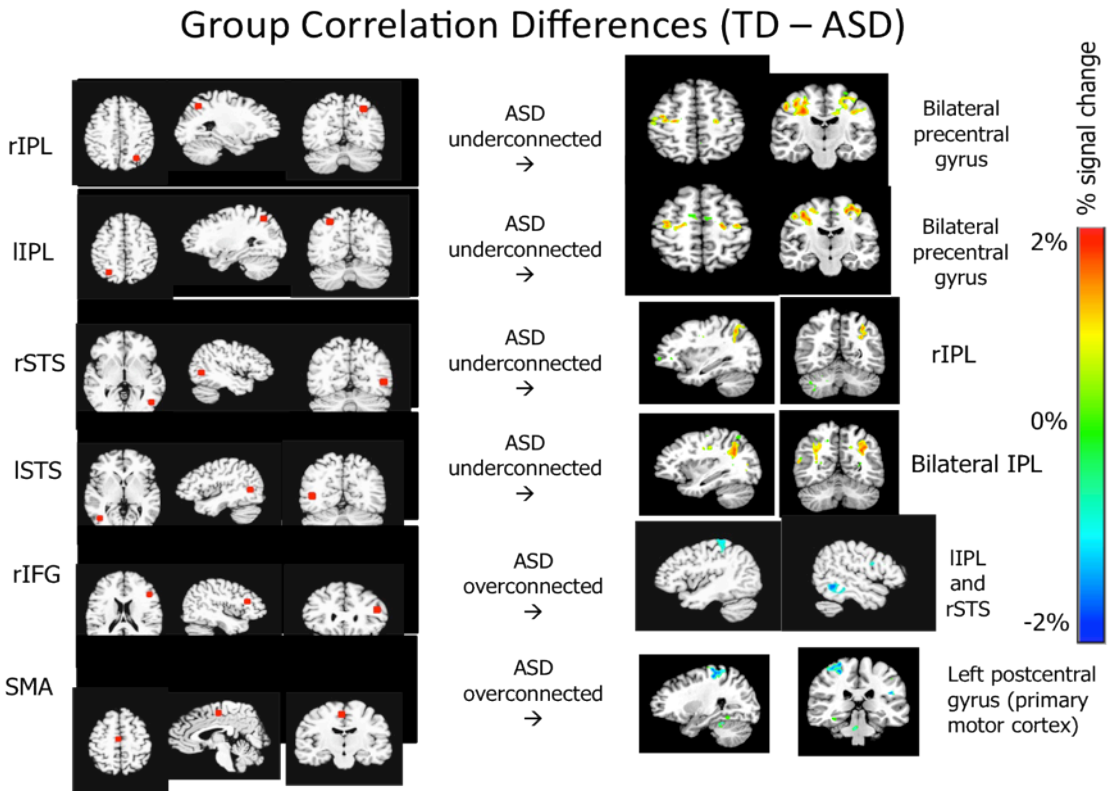


Fig. 5. Neurofeedback training increases activity in areas related to MNS

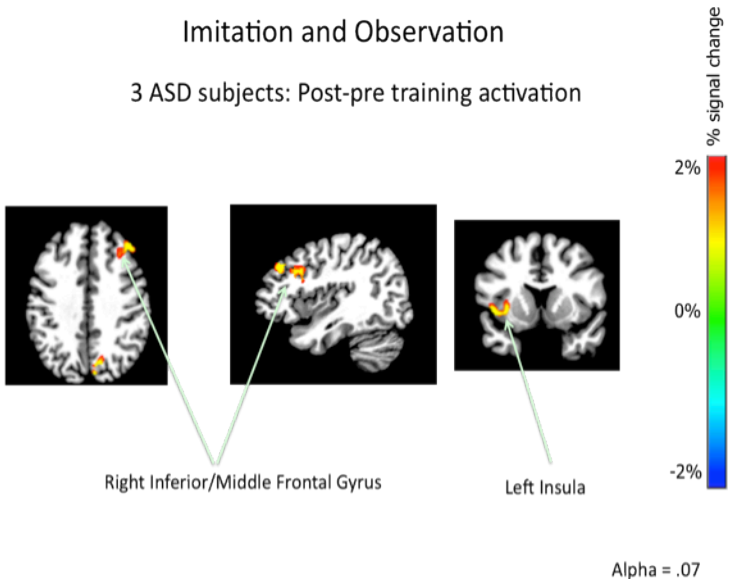
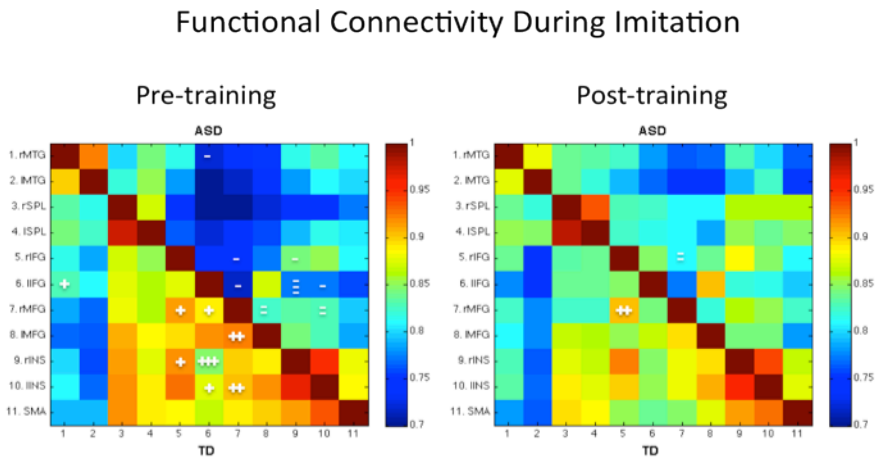


Fig. 6. Reduced abnormal connectivity is observed following the 20 weeks (30 hrs) of neurofeedback training in the ASD group.



Functional Neuroanatomical Changes Produced by Mu-based Neurofeedback Training in Children on the Autism Spectrum

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Abstract

It is widely agreed that the autistic brain is characterized by widespread aberrant connectivity that could underlie abnormal social behaviors. Nonetheless, the nature of the brain's experience-dependent plasticity suggests that these abnormal connection patterns may be reversed with the proper type of treatment. Indeed, neurofeedback training (NFT), an intervention based on operant conditioning resulting in self-regulation of brain electrical oscillations, has shown promise in addressing marked abnormalities in functional and structural connectivity. The effects of 30 hours of mu-rhythm based NFT on children on the autism spectrum (ASD), as well as typically developing (TD) children, were assessed behaviorally, electrophysiologically, and with fMRI. Prior to training, ASD participants showed significantly less activation compared to TD controls in an object-directed imitation task in areas associated with the human mirror neuron system (hMNS). Following training, ASD participants showed significantly greater activation in this task while TD participants did not. Prior to training, ASD participants also showed both over- and underconnectivity in resting state functional connectivity between areas of the hMNS compared to TD participants. These differences were significantly reduced following NFT. The fMRI changes accompanied improvements in behavioral and electrophysiological assessments in the ASD but not TD participants, indicating that the positive benefits shown to result from NFT are accompanied by modifications in functional neuroanatomy.

INTRODUCTION

Numerous findings support the theory that the social deficits in autism are the result of abnormal connectivity between brain regions associated with social cognition and action perception (*1*). The development of functional connectivity magnetic resonance imaging (fcMRI) has largely supported initial observations about neural connectivity derived from anatomical work (ref). Decreases in fcMRI in ASD are consistent across studies using various cognitive, emotional, and social tasks (*2-4*). Specific observations from these resting state fcMRI studies are that individuals with ASD show decreased resting state connectivity in the default mode network (DMN) compared to typically developing (TD) controls (*5*), as well as a reduced "switching" from this network to task-related networks during task performance (*6*). From these findings an underconnectivity hypothesis of ASD has emerged positing that "autism is a cognitive and neurobiological disorder marked and caused by under functioning integrative circuitry that results in a deficit of integration of information at the neural and cognitive levels" (*7*). A more general hypothesis has also been proposed suggesting that ASD may involve "local overconnectivity AND long-range underconnectivity" (*8*). That is, there appears to be more local connectivity within the primary visual cortex in autism, but there is less connectivity of this area of cortex to more distal sites such as the inferior frontal gyrus (*3*). Furthermore, individuals with ASD do not show as much deactivation, relative to controls, of the DMN when engaged in a task, a finding that indicates problems with regulation of functionally connected networks (*9*).

In particular, connectivity problems have been observed in brain regions constituting the human mirror neuron system (hMNS). The hMNS has provided a potential neurobiological substrate for understanding many key concepts in human social cognition directly relevant to the behavioral and cognitive deficits observed in ASD (*10*), including the ability to comprehend actions, understand intentions, and learn through imitation. First described in single-unit recordings by Rizzolatti and colleagues in the macaque monkey (*11*), mirror neurons are involved in both self-initiated action and the representation of action performed by others. Specifically, neurons in the pars opercularis of the inferior frontal gyrus (IFG) and in the inferior parietal lobule (IPL) show increased firing while executing *and* observing the same action, representing a potential mechanism for mapping seeing into doing (*12, 13*). Cells that fire preferentially in response to actions and to the observation of actions have also been observed in the human brain using single unit recordings (*14*). Indeed, a homologous network including the posterior superior temporal sulcus (pSTS) has been described in humans using fMRI (*15*).

Although some studies have raised questions about the role of mirror neurons in human social behavior (*16, 17*), an increasing amount of work suggests that a dysfunction in the hMNS does contribute to social deficits (*18-23*). Individuals with ASD have marked impairment in social skills, from joint attention to understanding the intentions of others or "mind-blindness" (*24, 25*), and as has been noted in a number of recent reviews, deficits in hMNS activity may explain the poor socialization skills prevalent in the disorder. A particularly striking fMRI study (*26*) demonstrated decreased activation in the pars opercularis of the IFG in autistic individuals, and found that activity in this region was inversely related to symptom severity in the social domain. EEG studies have also shown that

putative electro-biomarkers of hMNS activity show abnormalities in ASD compared to TD children (*20, 23, 27, 28*). Indeed, a body of evidence links the spectral dynamics of an EEG signal known as the mu rhythm to the functioning of the hMNS (*13*). Particularly relevant are scalp-recorded EEG patterns of activity in the alpha mu (8-13 Hz) and beta mu (15-25 Hz) range that are most evident over the central region of the scalp overlying the sensorimotor cortices and modulated by motor activity (*29*). Mu power is suppressed, relative to baseline, during the observation, execution, and imagination of body movements (*30-32*). Recently, Arnstein used simultaneous fMRI and EEG to show that suppression of mu power is correlated with BOLD signal activations in areas associated with the hMNS (*33*). In ASD individuals this mu rhythm suppression is not observed, supporting the role of an altered MNS in the disorder (*23, 34*).

We hypothesize that neurofeedback training (NFT) produces positive behavioral changes in ASD children and does this by normalizing the aberrant connections within and between neural circuits. Neurofeedback is a form of operant learning in which participants develop volitional control over the frequency-based spectral dynamics of scalp-recorded electrical cortical oscillations. Over time, participants develop strategies, implicitly or explicitly, to control a visual representation of frequency power levels. NFT requires less time than other behavioral interventions and produces many fewer side effects than pharmacotherapies (*35, 36*). In a recent review of the literature, Coben et al. (*37*) argued that while further research is necessary, a variety of studies support a Level 2 determination ("possibly efficacious") for the application of neurofeedback for autistic disorders. Previous mu-rhythm NFT in ASD children has led to improvements in measures of sociability and attention, and to a restoration of action-related mu-rhythm suppression that is normally absent (*38*). Therefore, it is hypothesized that lasting neuroplastic changes can result from repeatedly engaging circuits involved in developing and sustaining volitional control of cortical oscillations via NFT.

To test this hypothesis, we examined the effects of 30 hours of mu NFT administered in 45-minute sessions 2 times per week over the course of 20 weeks on a group of 9 high functioning ASD children (3 females), as well as a group of 10 typically developing (TD) children (3 females) between the ages of 8-17 yrs. The groups did not differ significantly by age (TD mean: 10.62, ASD mean: 12.88). Groups were also matched for handedness and intelligence (measured by the WASI). The ASD and TD groups were assessed before and after the training using multimodal neuroimaging as well as electrophysiological and behavioral assessments. To investigate the effects of training on functional activations of the hMNS, subjects performed an imitation task that was designed to elicit activity specifically in this system. We predicted that…..To investigate the effects of training on intrinsic functional connectivity, resting state scans were collected for all subjects. We predicted that ….During these scans, the participants underwent a functional MRI scan while letting their mind wander and not instructed to think about anything in particular. To investigate possible effects of training on white matter connectivity, diffusion tensor imaging scans were collected for all participants.

To investigate possible effects of training on behavioral and electrophysiological assessments, paper and pencil questionnaires and an MSI test were administered before and after training. We predicted that…. All of these neuroimaging measures, as well as behavioral and electrophysiological assessments, were collected for each participant's before and after training, so the effects of the training on each measure could be assessed.

We tested the first prediction by adapting an imitation task first used by Iacoboni et al. (1999). The major difference between the task used in the current study and the task used in previous studies is the use of a button box as the target of the imitated/observed action (Fig. 1A). By subtracting the activation that occurs during the static hand pictures from that which occurs during the finger movement videos, the activity that results from the visual features of the hand are eliminated and are left with the activity that results from observing and mirroring the finger motion itself. Likewise, by subtracting the activation that occurs during the spatial cue pictures from that which occurs during the finger movement videos, any activity that results from simple visuospatial reasoning is controlled for. Thus, our analysis used weighted contrasts where the average combined activity of the static hand and spatial cue stimuli is subtracted from the activity for the finger motion videos. This way, activity related to visual salience as well as simple spatial reasoning is controlled for, leaving only activity related to mapping observed movements onto one's own motor system (mirroring).

We found that for the imitation condition (execution during full motion video condition minus execution during picture/dot and blurred background/dot conditions), both groups activated hMNS areas of ventral premotor cortex, IPL, and STS. However, in the ASD group the only statistically significant activation was in STS. In the TD group, there was significant activation in STS as well as areas of the ventral premotor cortex (part of most models of hMNS). Direct group contrast (TD>ASD) in the imitation condition revealed voxel clusters where TD group showed significantly higher percent signal change relative to the ASD group (Fig 1B). These clusters were found in bilateral premotor cortex, rSTS, rIPL, and rIFG.

- Post-training Within-group results (pending)

The TD>ASD contrast for the post-training imitation condition found no clusters for which the TD group showed significantly greater activation than the TD group. This is quite different than the pre-training differences, which revealed many areas of ASD *hypo*activation. Interestingly, in this post-training contrast, there is now significantly greater activation in a cluster in the insula in the ASD group compared to the TD group (Fig. 2A). A contrast within the ASD group that examined post-training- minus pre-training activation revealed voxel clusters in which there was significantly greater activation for the imitation condition after the training compared to before (Fig. 2B). These clusters were found in bilateral inferior parietal lobule and bilateral insula. Additionally in this contrast, there were 2 areas that showed significantly reduced activity during the imitation condition after the training. These "negative" clusters were found in the ventral medial prefrontal cortex as well as the posterior cingulate cortex. These areas are part of the DMN (Fig. 2C). For the TD group, no clusters of significant difference were found after the training compared to before.

- Post-pre between-group: This analysis has not been performed yet, but will look for between-group differences in the amount of change from the pre- to the post-training scan. In other words, it will hopefully reveal areas in which the ASD group's post-training improvement was significantly greater than the TD group's post-training improvement.

For the functional connectivity analysis, regions of interest were determined based on areas showing the highest activation in the imitation task. Since these are different scans, it is not statistical double dipping to derive ROIs in this manner. FC analysis included white matter and ventricular signal regression. This step involves, for each individual run, extracting the BOLD signal time series in a region of interest containing only white matter or ventricular space. The following areas were used as seed regions of interest for the functional connectivity matrix analysis (they are written out here and each is followed by the abbreviation by which it will be referred for the remainder of this proposal): 1. Right superior temporal sulcus (rSTS), 2. Left superior temporal sulcus (lSTS), 3. Right superior parietal lobule (rSPL), 4. Left superior parietal lobule (lSPL), 5.Right inferior frontal gyrus (rIFG), 6. Left inferior frontal gyrus (lIFG), 7. Right medial frontal gyrus (rMFG), 8. Left medial frontal gyrus (lMFG), 9. Right insula (rINS), 10. Left insula (lINS), 11. Supplementary motor area (SMA).

In the pre-training resting state data between-group contrast of functional connectivity we found matrices revealing 4 network-node pairs that have significantly lower correlation in the ASD group compared to the TD group. These node-pairs (each corresponding to a single square on the connectivity matrix) included rSPL-lSPL, rMFG-rIFG, rINS-rIFG, and lINS-rIFG. In task-regressed runs there were between-group contrast of functional connectivity matrices with 9 node-pairs having significantly lower correlation in the ASD group compared to the TD group (Fig. 3A). These node-pairs included rINS-rSPL, rMFG-rIFG, rINS-rIFG, SMA-rIFG, lMFG-rMFG, SMA-rMFG, SMA-lMFG, SMA-rINS, and SMA-lINS. No significant group differences were found for any node-pairs on the resting state connectivity matrix following the training. This is in contrast with the underconnectivity observed in the ASD group prior to the training. In task-regressed runs all but one of the node-pairs that showed significant underconnectivity in the ASD group prior to the training was no longer statistically different from the TD group after the training. The node-pair that remained under connected after the training was rMFG-rIFG. Additionally, two node-pairs showed significant *over*connectivity in the ASD group following the training. These included rINS-lSTS and SMA-lSTS (Fig. 3B).

We tested the third prediction by….

We found ….

Behavioral and parental assessments

- Pre-training task-related behavioral measures
  - Reaction time

        - Pending
    - Accuracy
        - No group differences were found for response accuracy on the task.
- Post-training task-related behavioral measures
    - Reaction time
        - Pending
    - Accuracy
        - No group differences were found for response accuracy on the task after the training. Also, no differences were found between the post- and pre-training accuracy levels in either group.

(Fig. 4A)

We also investigated whether….

We found….

Electrophysiological (MSI) assessments

- Pre-training task-related
- Post-training task-related

(Fig. 4B)

Our findings have implications for operationalizing the benefits of NFT towards practical solutions to the early diagnosis and possible repair of hMNS deficits in autism. The best-established clinical application of the use of NFT and operant conditioning is arguably the treatment of epilepsy (*39*). Sterman and colleagues initially described an EEG oscillation with a frequency of 12–20 Hz, similar to EEG sleep spindles, which has been referred to as the "sensorimotor rhythm" or SMR (*40*). During the testing of a highly epileptogenic compound, Sterman and colleagues found elevated seizure thresholds in cats that had previously taken part in SMR conditioning, suggesting that the SMR training had somehow predisposed the cats against experiencing seizures. These findings have been successfully extrapolated to humans where it has been documented that seizure incidence is lowered significantly through SMR training (*41*). Consistent with the work by Sterman and colleagues, Ros et al. (*42*) have shown that self-regulation of EEG rhythms in quietly sitting, naive humans significantly affects the subsequent corticomotor response to transcranial magnetic stimulation (TMS), producing durable and correlated changes in neurotransmission. Finally, Beauregard and Lévesque J. et al. (*43*) scanned 15 unmedicated ADHD children randomly assigned to an experimental group that received NFT, and five other ADHD children assigned to the control group who did not receive NFT while they performed a Counting Stroop task. Prior to training, a significant focus of activation occurred in the left superior parietal lobule for both groups but no activation in the anterior cingulate cortex (ACC). Following training, there was still increased activation of the left superior parietal lobule for both groups, but for the experimental group only there was a significant activation of the right ACC. Our findings,

therefore, support the idea that studying the patterns of aberrant connectivity in ASD is a valuable step in understanding how neurological factors may account for the behavioral profiles of individuals with ASD. It is also a means to assess the effectiveness of interventions, including NFT.

The strongest post-training finding (statistically speaking) in the fMRI data was increased bilateral insula activation in the ASD group. Numerous models of the core and extended human mirror neuron system include the insula as a critical hub of this network (*44*). This area is important for integrating external perceptions and stimuli with internal representations and interoception. It plays a role in mediating empathic responses to facial expressions and social interactions (citation). It seems to be involved in mediating, and is possibly responsible for generating, the actual subjective experience of basic human emotions such as disgust and happiness (citation). In a recent review (*45*) it was shown that insula dysfunction is a recurring finding in fMRI studies of individuals with ASD. Many studies report reduced activation of insula in ASD groups, as well as reduced functional connectivity to and from this area. Recently, Ebisch et al (*46*) showed that autistic individuals had reduced functional connectivity between both the anterior and posterior insula and areas involved in processing of social and affective stimuli. It is compelling evidence that mu-based NFT is strengthening connections and improving neurophysiological function in an area that shows marked dysfunction in ASD.

The other cluster in which there was significantly higher activation in the ASD group after the training period was found in bilateral IPL. This structure plays a significant role in sensorimotor integration and is one of the defining areas of the core human mirror neuron system. IPL receives input from the visual system via the STS. It feeds input into the ventral premotor cortex and in particular the IFG. The IFG does not receive direct visual input, and thus IPL acts as a waypoint in this pathway. Dysfunction in this area has the potential to cause widespread deficits.

The finding of increased task-related deactivation of the default mode network (DMN) after NFT was not explicitly predicted but makes sense in light of studies showing DMN abnormalities in ASD. The DMN normally exhibits task-related deactivation, with concurrent increases in task-positive networks. Kennedy et al observed that ASD participants show less task-related deactivation of the DMN compared to TD controls (*6, 47*). The finding of the present study with regards to the default mode network suggests that NFT may be allowing the brain to switch more readily between task-positive and task-negative networks when such a switch is appropriate.

Interestingly, the increases in functional activations after training in the ASD group are not seen in the TD group. At the same statistical threshold, a comparison of the post-training TD group activation during the imitation condition compared to that of the pre-training TD group activation revealed no statistically significant clusters of activation differences. This suggests that the effects observed in the ASD group are appearing specifically because of the normalization of components of the core and extended mirror neuron system, including the bilateral inferior

parietal lobule and the bilateral insula, via neurofeedback training. Thirty hours of mu-based neurofeedback training apparently does not significantly alter connections in an already "intact" mirror system.

Importantly, we also observed behavioral and electrophysiological changes after the training that correlated positively with increased activations in the imitation-observation task. Also, since the electrophysiological effects of NFT were the focus of a previous study, this component of the study was a replication of previous results. Basically, prior to the training, mu suppression was absent in the ASD group in response to action observation. Following the training, the ASD group recovered this mu suppression, and their overall improvement in this measure was significantly greater than the TD controls who underwent the same training. The same effect was seen for behavioral assessments. ASD children showed improvements on parental assessment measures of social behavior and communication, and these improvements were greater than those seen in the TD group. The fact that behavioral increases are correlated with increased task-related brain activations suggests that NFT is causing behavioral improvements by way of changing the dynamics of brain networks, and in particular the hMNS.

Williams et al (*48*) showed that during performance of an imitation task, activation of the IPL, as well as the STS, was lower for ASD participants in the imitation vs. other execution (spatial/static cue) contrast. In addition, they found areas of ventral prefrontal cortex that had higher activation in the ASD group for this contrast. Therefore, not only has this imitation task been shown to reliably produce BOLD activations in the regions associated with the human mirror neuron system (*15*), but it has also been shown to elicit differential activations in the MNS of autistic patients compared to healthy controls. However, Williams et al (*48*) did not show differences in activation between groups in the imitation vs. static/spatial cue conditions in the IFG, one of the key areas of the human mirror neuron system.

The major difference between the task used in the current study and the task used in previous studies is the use of a button box as the target of the imitated/observed action. The inclusion of the button box effectively changes the task from an imitation of relatively meaningless finger movement to an imitation of object-directed movement. Evidence suggests that the human mirror neuron system, and the inferior frontal gyrus (IFG) in particular, responds preferentially to object-oriented action (*31, 49*). Also, the human IFG may be an anatomical homologue to area F5 of the premotor cortex in monkeys, which responds preferentially to object-related movements and grasps. Thus, while meaningless action would most likely still elicit some degree of MNS activation, object-directed action may produce stronger BOLD signal activation during the task. This may have the effect of making our task more sensitive to between-group differences in imitation-related activation. It is worth noting that the current task, like the Iacoboni and Williams's task, requires that participants perform mirror-image imitation. That is, they are using their right hand to imitate a movement that they see being performed with a left hand, as if they were looking in a mirror. Studies suggest that there is a selective deficit for mirror-image imitation in autism (*50*). Another advantage of the inclusion of the button-box is that, since the press of a button is an easily quantifiable response,

it allows me to compare accuracy (as measured by the number of correct button presses during execution trials) within and between groups.

An obvious limitation to this data is the relatively small sample size. However, the results are surprisingly strong and clear in light of this caveat. In addition, I propose several other types of analysis to be performed on the data obtained from this study. One important step will be to assess the degree of correlation between symptom severity and the magnitude of behavioral/neural response to NFT. This type of analysis will address the issue of whether mu neurofeedback is more beneficial for individuals with more severe symptoms of ASD. Another important question is whether those individuals with the most dramatic behavioral and parental assessment improvements also show the greatest changes in functional neurophysiological activation during the imitation task.

Could the functional changes observed here eventually cause lasting structural changes in white matter tracts? Evidence suggests that there are widespread abnormalities in structural integrity of WM tracts in ASD (citations???). The present study has included the collection of diffusion tensor imaging (DTI) data for all subjects, pre- and post-training. DTI is an MRI-based brain imaging method that can provide information about the structural integrity and connectivity within and between white matter fiber tracts in the brain. One DTI analysis technique that I propose to use on the datasets I obtain is known as probabilistic tractography. Technically speaking, this algorithm works by finding primary, secondary and tertiary eigenvectors of diffusion occurring in each voxel. Using this information, it can identify tracts that xxx.

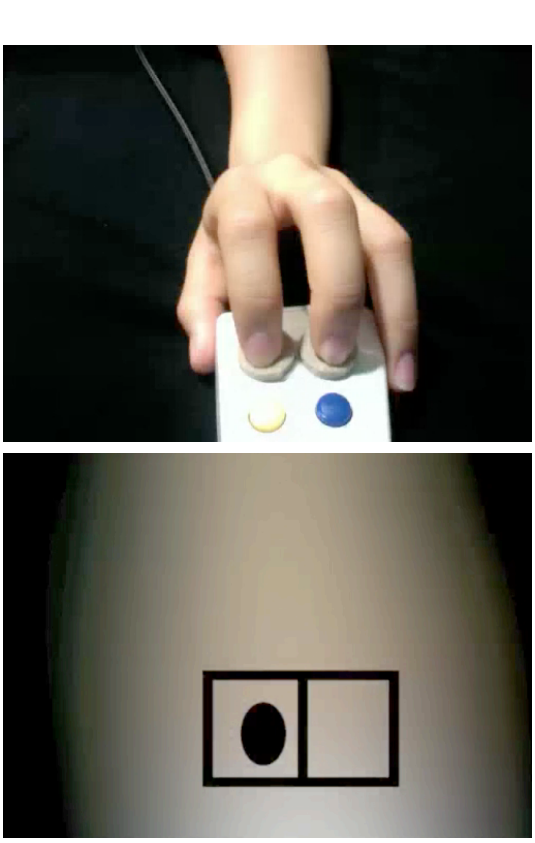

Figure 1. Example stimuli for the hand motion video (top left), static hand image (top right), and blurred spatial (bottom) cue conditions.

Reference List

1. R. A. Muller, *Ment. Retard. Dev. Disabil. Res. Rev.* **13**, 85 (2007).
2. M. A. Just, S. Varma, *Cogn Affect. Behav. Neurosci.* **7**, 153 (2007).
3. M. E. Villalobos, A. Mizuno, B. C. Dahl, N. Kemmotsu, R. A. Muller, *Neuroimage* **25**, 916 (2005).
4. D. E. Welchew *et al.*, *Biol. Psychiatry* **57**, 991 (2005).
5. V. L. Cherkassky, R. K. Kana, T. A. Keller, M. A. Just, *Neuroreport* **17**, 1687 (2006).
6. D. P. Kennedy, E. Courchesne, *Soc. Cogn Affect. Neurosci.* **3**, 177 (2008).
7. M. A. Just, V. L. Cherkassky, T. A. Keller, N. J. Minshew, *Brain* **127**, 1811 (2004).
8. E. Courchesne, K. Pierce, *Curr. Opin. Neurobiol.* **15**, 225 (2005).
9. D. P. Kennedy, E. Redcay, E. Courchesne, *Proc. Natl. Acad. Sci. U. S. A* **103**, 8275 (2006).
10. J. H. Williams, A. Whiten, T. Suddendorf, D. I. Perrett, *Neurosci. Biobehav. Rev.* **25**, 287 (2001).
11. G. di Pellegrino, L. Fadiga, L. Fogassi, V. Gallese, G. Rizzolatti, *Exp. Brain Res.* **91**, 176 (1992).
12. G. Rizzolatti, L. Craighero, *Annu. Rev. Neurosci.* **27**, 169 (2004).
13. J. A. Pineda, *Brain Res. Brain Res. Rev.* **50**, 57 (2005).
14. R. Mukamel, A. D. Ekstrom, J. Kaplan, M. Iacoboni, I. Fried, *Curr. Biol.* (2010).
15. M. Iacoboni *et al.*, *Science* **286**, 2526 (1999).
16. G. Hickok, *J. Cogn Neurosci.* **21**, 1229 (2009).
17. L. Turella, A. C. Pierno, F. Tubaldi, U. Castiello, *Brain Lang* **108**, 10 (2009).
18. N. Nishitani, S. Avikainen, R. Hari, *Ann. Neurol.* **55**, 558 (2004).
19. N. Hadjikhani, R. M. Joseph, J. Snyder, H. Tager-Flusberg, *Cereb. Cortex* **16**, 1276 (2006).
20. R. Bernier, G. Dawson, S. Webb, M. Murias, *Brain Cogn* **64**, 228 (2007).
21. M. Dapretto *et al.*, *Nat. Neurosci.* **9**, 28 (2006).
22. H. Theoret *et al.*, *Curr. Biol.* **15**, R84 (2005).
23. L. M. Oberman *et al.*, *Cognitive Brain Research* (2005).

24. M. Carpenter, K. Nagell, M. Tomasello, *Monogr Soc. Res. Child Dev.* **63**, i (1998).
25. S. Baron-Cohen, *Ann. N. Y. Acad. Sci.* **1156**, 68 (2009).
26. M. Dapretto *et al.*, *Nat. Neurosci.* **9**, 28 (2006).
27. L. M. Oberman, V. S. Ramachandran, J. A. Pineda, *Neuropsychologia* (2008).
28. R. Raymaekers, J. R. Wiersema, H. Roeyers, *Brain Res.* **1304**, 113 (2009).
29. E. L. Altschuler, A. Vankov, V. Wang, V. S. Ramachandran, J. A. Pineda, *J. Cogn Neurosci.* (1998).
30. G. Pfurtscheller, C. Brunner, A. Schlogl, F. H. Lopes da Silva, *Neuroimage.* **31**, 153 (2006).
31. S. D. Muthukumaraswamy, B. W. Johnson, N. A. McNair, *Brain Res. Cogn Brain Res.* **19**, 195 (2004).
32. D. J. McFarland, L. A. Miner, T. M. Vaughan, J. R. Wolpaw, *Brain Topogr.* **12**, 177 (2000).
33. D. Arnstein, F. Cui, C. Keysers, N. M. Maurits, V. Gazzola, *J. Neurosci.* **31**, 14243 (2011).
34. L. M. Oberman, V. S. Ramachandran, *Psychol. Bull.* **133**, 310 (2007).
35. T. Fuchs, N. Birbaumer, W. Lutzenberger, J. H. Gruzelier, J. Kaiser, *Appl. Psychophysiol. Biofeedback* **28**, 1 (2003).
36. D. J. Fox, D. F. Tharp, L. C. Fox, *Appl. Psychophysiol. Biofeedback* **30**, 365 (2005).
37. R. Coben, S. Sherlin, W. J. Hudspeth, K. McKeon, *Journal of Autism and Developmental Disorders* (2009).
38. J. A. Pineda *et al.*, *Research in Austism Spectrum Disorders* **2**, 557-581 (2008).
39. M. B. Sterman, *Biofeedback Self Regul.* **21**, 3 (1996).
40. S. R. Roth, M. B. Sterman, C. D. Clemente, *Electroencephalogr. Clin. Neurophysiol.* **23**, 509 (1967).
41. J. E. Walker, *Clin. EEG. Neurosci.* **39**, 203 (2008).
42. T. Ros, M. A. Munneke, D. Ruge, J. H. Gruzelier, J. C. Rothwell, *Eur. J. Neurosci.* **31**, 770 (2010).
43. M. Beauregard, J. Levesque, *Appl. Psychophysiol. Biofeedback* **31**, 3 (2006).
44. J. A. Pineda, *Behav. Brain Funct.* **4**, 47 (2008).
45. L. Q. Uddin, V. Menon, *Neurosci. Biobehav. Rev.* **33**, 1198 (2009).
46. S. J. Ebisch *et al.*, *Hum. Brain Mapp.* **32**, 1013 (2011).
47. M. Assaf *et al.*, *Neuroimage.* **53**, 247 (2010).
48. J. H. Williams *et al.*, *Neuropsychologia* **44**, 610 (2006).
49. S. H. Johnson-Frey *et al.*, *Neuron* **39**, 1053 (2003).
50. S. Avikainen, A. Wohlschlager, S. Liuhanen, R. Hanninen, R. Hari, *Curr. Biol.* **13**, 339 (2003).